A Dose-Response Study of a Live Attenuated Varicella-Zoster Virus (Oka Strain) Vaccine Administered to Adults 55 Years of Age and Older

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Decreased cell-mediated immune (CMI) response to varicella-zoster virus (VZV) is correlated with an increased risk of reactivation of latent virus from dorsal root sites, leading to herpes zoster. The cell-mediated and humoral immunogenicity of three concentrations (3200, 8500, and 41,650 pfu/dose) of a live attenuated VZV vaccine (Oka strain; VZV/Oka) was compared with a control pneumococcal polysaccharide vaccine in 200 healthy adults who were ⩾55 years old. Six weeks after vaccination, the VZV-specific CMI response (as measured by stimulation index values and precursor cell frequencies) was enhanced in all VZV/Oka vaccine groups compared with the control group (for all VZV/Oka groups combined vs. controls, tested with VZV crude antigen: stimulation index, $P < .001$; precursor cell frequency, $P < .001$). Geometric mean titers of anti-VZV antibodies increased in all VZV/Oka vaccine groups but remained unchanged in the control vaccine group. No dose effect of VZV/Oka vaccine was observed for CMI or humoral responses.

Herpes zoster is a painful cutaneous eruption caused by reactivation of latent varicella-zoster virus (VZV), the agent that causes chickenpox in children. It is estimated that over a lifetime, ~15% of persons who experienced clinical or subclinical varicella infection will eventually develop zoster [1]. Herpes zoster may be associated with severe neurologic manifestations and complications, mainly acute pain and postherpetic neuralgia.

The risk of developing herpes zoster is inversely correlated to the status of VZV-specific cell-mediated immunity. Progressive loss of cell-mediated immunity is a natural part of the aging process and is correlated with an increase of zoster incidence and more severe zoster complications (e.g., postherpetic neuralgia and ophthalmic zoster) [1–3].

Enhancement of the VZV-specific immune response of healthy adults by immunization with a live attenuated VZV vaccine (Oka strain; VZV/Oka) was first demonstrated by Berger et al. [4]. Subsequently, these results were confirmed by Levin et al. [5], who immunized a cohort of 202 healthy older subjects (⩾55 years) with one or two doses of a live attenuated VZV/Oka vaccine at different concentrations (1140 to 12,040 pfu/dose) and demonstrated that both humoral and cell-mediated VZV immunity could be boosted by active immunization with VZV/Oka. Moreover, the 4-year follow-up of this cohort suggested that vaccination with VZV/Oka may decrease the severity of zoster episodes [6]. Of note, none of these previous studies included a control group.

Vaccination with live attenuated VZV vaccine thus represents a potential strategy for preventing zoster and/or preventing or lessening zoster-associated pain in persons who may already harbor latent VZV [7]. This randomized, controlled trial in 200 healthy older adults evaluated the cell-mediated and humoral immunogenicity and the safety of one of three doses of a live attenuated VZV/Oka vaccine compared with a control vaccine.

Materials and Methods

Study population. Healthy adults were enrolled in this study during routine out-patient visits. The main inclusion criteria were age ⩾55 years, previous history of varicella confirmed by positive serology to VZV (screened using Enzygnost; Behringwerke, Marburg, Germany), and a competent immune system (no signs of immunodeficiency). Main exclusion criteria were fever at the time of selection, any previous zoster episode, seropositivity to human immunodeficiency virus (screened using Genelavia mixt; Sanofi Diagnostics Pasteur, France), any underlying immunodepressive condition, previous vaccination against varicella or zoster, any other recent vaccination, recent administration of any blood product, and sensitivity to neomycin.

Study design. Study participants were randomly assigned to 1 of 4 vaccine groups. Three groups received a subcutaneous injection of a live attenuated VZV/Oka vaccine [8] manufactured by Pasteur Mérieux Connaught (Lyon). Patients in these groups were randomly allocated one of three concentrations (3200, 8500, or 41,650 pfu/dose) of live VZV/Oka under double-blind conditions. The fourth group was vaccinated subcutaneously with a licensed pneumococcal polysaccharide vaccine (Pneumo 23, lot L0042; Pasteur Mérieux Connaught, Lyon; distributed in France by Pasteur Mérieux Sérums et Vaccins, Marnes-la-Coquette). This vaccine was administered under single-blind conditions and was used as a control for reactogenicity and immune response.

Informed consent was obtained from all subjects who participated in this study. The trial was approved by the Ethics Committee of the University Hospital, Basel, Switzerland.

Financial support: Pasteur Mérieux Connaught, Lyon, France.

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The Journal of Infectious Diseases 1998; 178(Suppl 1):S99–103

0022–1899/98/78S1–0022$02.00

Vaccine recipients noted the occurrence of any adverse reactions to vaccination in individual monitoring diaries and were followed by means of medical visits at days 3 and 42 after vaccination. If vesicular rash or zoster occurred, subjects returned to the study center for an additional medical visit, and vesicles were sampled for VZV identification by polymerase chain reaction (PCR) and restriction analysis [9].

Vaccine. The Pasteur Mérieux Connaught VZV/Oka vaccine is freeze-dried and has been demonstrated to have good stability for at least 2 years at 5°C [10]. The vaccine should be reconstituted with diluent (NaCl, 4 g/L) immediately before subcutaneous injection. Three lots corresponding to the three concentrations were used for the study: S2885, 3200 pfu/dose; S2887, 8500 pfu/dose; and S3061, 41,650 pfu/dose. These concentrations of VZV/Oka vaccine were chosen in order to obtain widely differing virus titers between the 3 groups (i.e., a difference of at least 0.3 in $\log_{10}$ plaque-forming units [pfu]). The control, a purified polyvalent *Streptococcus pneumoniae* polysaccharide liquid vaccine, was administered subcutaneously.

Assays for VZV immunity. Blood samples were obtained for the measurement of cell-mediated immune (CMI) and humoral immune responses before and 42 days after vaccination.

T cell recognition of VZV antigen was determined by incubating peripheral blood mononuclear cells (10^5/well) with dilutions of inactivated crude VZV antigen (Pasteur Mérieux Connaught, Lyon), purified VZV glycoproteins (Viro Research, Rockford, IL), purified VZV immediate early protein 62 (IE62; Viro Research), and MRC-5 substrate antigens (negative control) and tetanus toxoid (positive control) (Pasteur Mérieux Connaught, Lyon) in RPMI medium with 10% human serum for 6 days [5, 11]. The inactivated VZV crude antigen and MRC-5 antigen were each prepared at protein concentrations of 3.5 mg/mL. All antigen dilutions were made with lymphocyte culture medium. Optimal antigen concentrations in cultures were 1:3000 of the initial solution for inactivated VZV crude antigens and MRC-5 antigen (precise quantitation of antigen was not possible), 417 ng/mL for VZV glycoproteins, 167 ng/mL for IE62, and 50 μg/mL for tetanus toxoid. Proliferation was detected by [^{3}H-methyl]thymidine uptake during 6 h of incubation.

The stimulation index (SI) was calculated as the ratio of the mean counts per minute (cpm) in antigen-specific stimulated wells to the mean cpm in the respective negative control wells. Four replicates per measurement were performed.

The precursor cell frequency (PCF) analysis was done by use of the limiting dilution culture as described by Levin et al. [5] and Hayward et al. [11], except that cultures were incubated for 6 rather than 10 days. PCF was expressed as the number of VZV antigen–specific cells per 10^6 lymphocytes.

Humoral immune response. Determination of IgG antibodies to VZV were measured before vaccination and 6 weeks after vaccination (day 42) using an in-house glycoprotein ELISA [12] at Pasteur Mérieux Connaught (Laboratoire Séro-Immuno Clinique, Val de Reuil, France).

Data analysis. Data were dual entered in database software (Climed; Simed, Créteil, France). Statistical analysis was done by the Biometry Department of Pasteur Mérieux Connaught (Marnes-la-Coquette) using SAS software (SAS Institute, Cary, NC); SI values and PCFs were estimated before vaccination and at day 42. For the purposes of analysis, the PCF at the upper limit of detection was taken to be $500/10^6$ cells. After $\log_{10}$ transformation, geometric means of the SI and PCF were calculated for each vaccine group. Since statistical tests did not reject the homogeneity of the regression lines for pre- and postvaccination responses between the 4 vaccine groups (slope test: $P \geq .184$ for SI and PCF), data were analyzed by use of a covariance analysis model. This statistical procedure enabled adjustment for prevaccination values. The following comparisons were made: all VZV/Oka vaccine groups against control, highest dose of VZV/Oka vaccine against control, and trend effect for the different VZV/Oka doses (dose response). Contrasts were done at the 2.5% level of significance, adjusting for three related contrasts.

Table 1. Percentage of subjects ($n = 200$) with local adverse reactions during 6 weeks after vaccination with Oka VZV/Oka vaccine, according to vaccine group.

	VZV/Oka vaccine			
Injection-site reaction	3200 pfu	8500 pfu	41,650 pfu	Pneumo 23* vaccine
None	60.0	58.8	73.5	34.0
⩾1 reaction	40.0	41.2	26.5	66.0
Induration (diameter ⩾2 cm)	18.0	21.6	8.2	16.0
Pain (all)	28.0	37.2	24.5	58.0
Pain (probably vaccine related)	14.0	19.0	12.0	29.0
Redness (diameter ⩾2 cm)	26.0	17.6	12.2	26.0
Pruritus	10.0	7.8	8.2	6.0
Vesicle	0	2.0	0	0

* Pneumococcal polysaccharide vaccine (licensed by Pasteur Mérieux Connaught, Lyon, France, and distributed in France by Pasteur Mérieux Sérums et Vaccins, Marnes-la-Coquette).

Results

Study population. Two hundred older adult subjects (age range, 55–88 years), comprising 82 women and 118 men, were enrolled in the trial. Slightly fewer women (34%) and a higher mean age (67.7 years) were found in the 3200-pfu VZV/Oka vaccine group compared with the other groups (women, 41%–45%; mean age, 64.7–65.2 years); however, the higher age in this group can be explained by the advanced age (77 and 88 years) of 2 members. Although a possible potential selection bias cannot be ruled out, between-group differences were mostly likely random, and the age and gender distributions were not considered different among the 4 groups.

Reactogenicity and safety. Adverse reactions observed during the 6 weeks following vaccination are summarized in table 1. Overall, the VZV/Oka vaccine was well tolerated compared with the pneumococcal polysaccharide control vaccine. At least one adverse reaction was experienced by 26.5%–41.2% of the subjects in the groups that received one of the doses of the VZV/Oka vaccine, compared with 66% of subjects who received the control vaccine. Pain at the injection site that was judged by the investigator to be probably related to the vaccine

was reported less frequently after VZV/Oka vaccination (12%–19% of subjects) than after the pneumococcus control vaccination (29%). Incidences of redness, pruritus, and induration were no more frequent in the VZV/Oka groups than in the control group.

No subject had a fever during the 72 h following vaccination. One subject in the 8500-pfu VZV/Oka group presented with a mild vesicular rash at the injection site (<10 vesicles) that occurred 1 day after vaccination and lasted 7 days. PCR analysis of the vesicular fluid was negative for VZV.

CMI response to VZV. Analysis of the geometric mean of SI values showed that the VZV antigen–specific proliferative response was significantly enhanced in all VZV/Oka vaccine groups after vaccination but remained unchanged in the control group. On day 42, the mean proliferative responses in the groups receiving VZV/Oka vaccine, adjusting for prevaccination titers, were 5.62–7.24 for crude VZV antigen (vs. 2.88 in the controls), 2.29–3.09 for purified VZV glycoproteins (vs. 1.41 in controls), and 1.38–1.58 for IE62 (vs. 1.05).

SI ratios (day 42/before vaccination) and 95% confidence intervals (95% CIs) were 1.6 (95% CI, 1.1–2.2), 1.5 (95% CI, 1.1–2.1), and 2.5 (95% CI, 1.9–3.3) in the 3200-pfu, 8500-pfu, and 41,650-pfu groups, respectively, compared with 0.85 (95% CI, 0.6–1.2) in the control group. A covariance analysis model with prevaccination SI values as covariables did not detect any statistically significant dose effect for the different VZV/Oka concentrations in the CMI response to VZV, as measured by the adjusted geometric mean SI at day 42 (trend test not significant for any VZV antigen: trend effect $P \geq .177$). Nonetheless, considering all VZV/Oka groups together, adjusted geometric mean SI values were statistically significantly higher after VZV/Oka vaccination than control vaccination for all VZV antigens ($P < .001$, crude VZV; $P < .001$, VZV glycoproteins; $P < .003$, IE62).

The PCFs before and after (day 42) vaccination are presented in figure 1. Geometric mean PCF (per 10^6 cells) increased after vaccination in all VZV/Oka groups but remained unchanged in the control group. Increases in geometric mean PCFs (per 10^6 cells) from before vaccination to day 42 were as follows for the different VZV/Oka doses: from 52.5 to 89.1 in the 3200-pfu group, from 37.2 to 91.2 in the 8500-pfu group, and from 22.4 to 66.1 in the 41,650-pfu group. In the control group, the geometric mean PCF decreased slightly from 37.2 before vaccination to 26.9 at day 42. This very small decrease does not appear related to the pneumococcal polysaccharide control vaccine since the same effect was also observed with the control tetanus toxoid antigen (data not shown). After adjustment for prevaccination PCFs, no statistically significant dose effect was found for the different VZV/Oka doses in the geometric mean PCF at day 42. Still, the adjusted geometric mean PCF was statistically significantly higher when all VZV/Oka groups together were compared against the control group.

Humoral immune response. After vaccination, geometric mean titer values of antibodies to VZV glycoproteins increased in all VZV/Oka vaccine groups and remained unchanged in the pneumococcal polysaccharide control vaccine group. The geometric mean titer ratios (day 42/before vaccination) and 95% CIs were 1.4 (95% CI, 1.2–1.7), 1.4 (95% CI, 1.2–1.7), and 1.3 (95% CI, 1.2–1.7) in the 3200-pfu, 8500-pfu, and 41,650-pfu groups, respectively, compared with 1.0 (95% CI, 0.9–1.1) in the control group. Increases were significant in all VZV/Oka groups (lower boundary of 95% CIs, >1.0), but not in the control group (lower boundary of 95% CIs, <1.0).

A correlation analysis revealed that the antibody response was not correlated with the SI response, irrespective of the VZV antigen used ($r = .05$, Pearson's correlation coefficient).

Discussion

The results from this randomized, controlled study confirmed previous observations in uncontrolled trials that vaccination with live attenuated VZV/Oka vaccine is safe and enhances CMI to VZV in older adult subjects [4, 5]. The VZV/Oka vaccine used in this study was well tolerated, and vaccinated subjects presented few local and general reactions, even with the highest dose of VZV/Oka vaccine used (41,650 pfu). In fact, the incidence of local reactions was lower in the group of subjects who received the highest dose of VZV/Oka vaccine (26.5%) compared with the other 2 VZV/Oka groups (3200 pfu, 40.0%; 8500 pfu, 41.2%), although these differences were not tested statistically. In particular, induration was reported less frequently in the 41,650-pfu group (8.2% compared with 18.0%–21.6%), and redness tended to decrease as the vaccine dose increased (26.0%–12.2%). The reason for these descriptive differences between the different doses of VZV/Oka vaccine is not clear but might be due to chance or other unknown factors, since the safety evaluation was done under double-blind conditions. Of importance, the local reactogenicity of each dose of VZV/Oka was better than that of the widely used pneumococcal polysaccharide control vaccine.

To our knowledge, this is the first time such a high vaccine dose (41,650 pfu) has been administered to VZV-seropositive subjects, and it confirms the innocuous nature of the VZV/Oka strain. Only 1 subject (in the 8500-pfu group) presented postvaccination vesicles (<10), which might have been caused by the vaccine; however, the vesicles were VZV-negative as determined by PCR analysis of vesicular fluid. No subject presented any varicella-like rash or zoster during the 6-week follow-up.

All in vitro measurements of cell-mediated immunity (SI and PCF) and VZV glycoprotein–specific antibody titers were significantly increased 6 weeks after vaccination (day 42) in the 3 VZV/Oka groups, compared with the control group. The adjusted geometric means for SI values at day 42 were about two times higher in the VZV/Oka groups compared with the control group, and, likewise, adjusted geometric mean PCFs were approximately three times higher in the active vaccine groups. Results were consistent for all VZV antigens, but, as

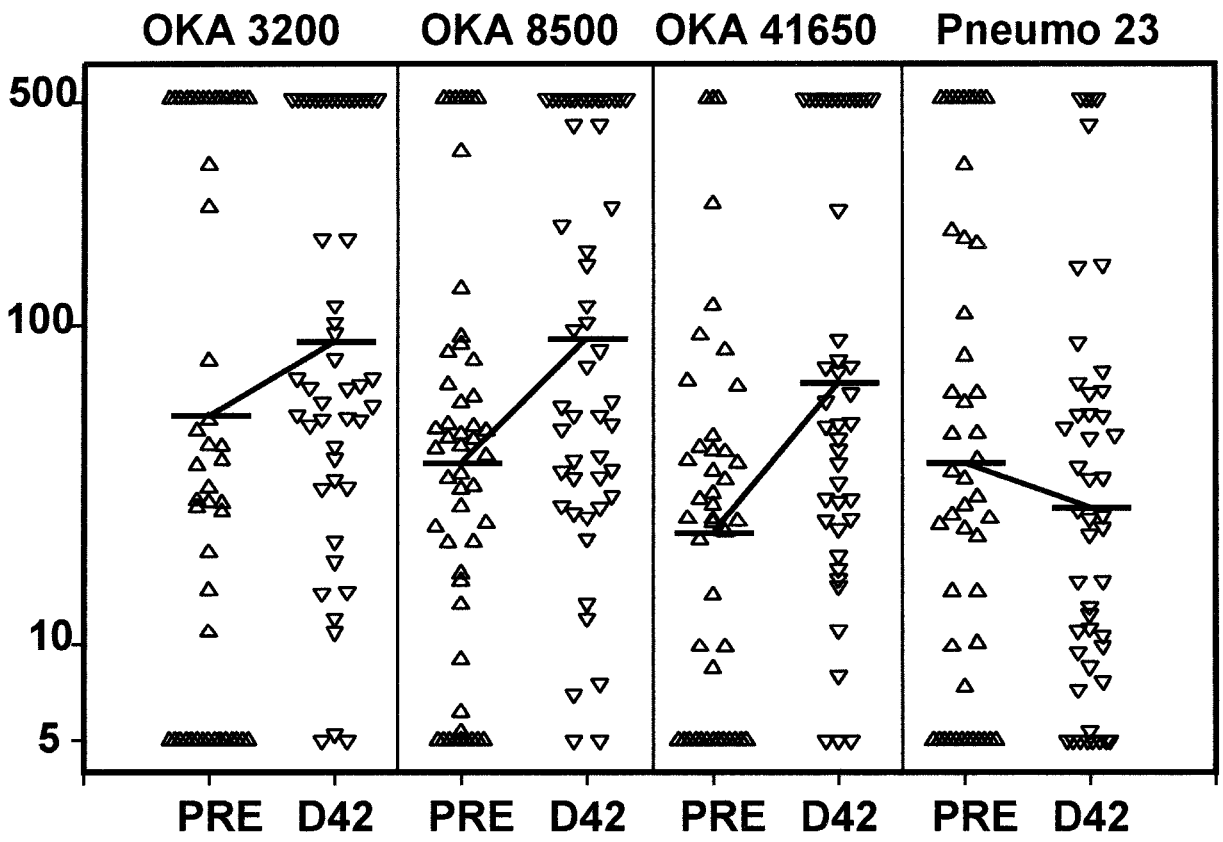

Figure 1. Individual precursor cell frequencies (PCFs; no./10^6 cells) in 4 groups of subjects before (PRE) and 42 days after (D42) vaccination with various doses (in plaque-forming units) of Oka strain of VZV or with Pneumo 23 (pneumococcal polysaccharide vaccine; Pasteur Mérieux Connaught, Lyon, France; distributed in France by Pasteur Mérieux Sérums et Vaccins, Marnes-la-Coquette). Solid horizontal bars = geometric mean PCFs.

expected, crude VZV antigen elicited the best proliferative response. Proliferative response to purified IE62 VZV antigen was observed only in a minority of vaccinees, and all of these responders to IE62 demonstrated a proliferative response to VZV glycoproteins. Furthermore, all responders to VZV glycoproteins demonstrated a response to crude VZV antigen.

Six weeks after vaccination, there were no significant differences in VZV-specific immune responses between the different virus concentrations tested. This indicated that either the lowest dose (3200 pfu) is sufficient to boost the CMI or that other inactive viral components of the VZV/Oka preparation used may compensate for the lower pfu content by additionally enhancing the immune response [13]. As previously reported by Levin et al. [5], the VZV CMI response early after vaccination was not related to the dose of the vaccine given. However, a difference may appear between doses after several years of follow-up, with a longer-lasting enhancement of immune response being obtained with high vaccine doses [6]. Further long-term assessment of CMI and humoral response after vaccination with VZV/Oka vaccine will be needed to confirm this latter finding.

Although prevaccination VZV antibody titers tended to be high in the subjects, this did not prevent vaccination with VZV/Oka from boosting the CMI and humoral immune responses. Six weeks after vaccination with VZV/Oka vaccine, VZV-antibody levels had increased by ~30%–40%, and a significant proliferative response was obtained. No boost of VZV glycoprotein–specific antibodies or CMI response was seen in the control group.

The vaccine concentrations tested were selected on the basis of infectivity titer (pfu/dose), not on the inactive viral antigen. The three concentrations were chosen to obtain significantly different pfu contents per dose, according to the accuracy of the plaque assay. On the basis of our data, the lowest dose of VZV/Oka vaccine (3200 pfu) is sufficient to significantly boost both CMI and antibody response to VZV.

A long-term follow-up of the vaccinated subjects is being conducted both to determine whether the enhanced VZV-specific CMI response obtained after a single vaccination with VZV/Oka is long lasting and also to monitor the zoster incidence. It is not known whether a boost of VZV cell-mediated immunity will protect against the occurrence of zoster or, as

seems more likely, will lessen the severity of a zoster episode [6, 7]. A large, double-blind, placebo-controlled trial is needed to assess the efficacy of this vaccine in the prevention or reduction of the severity of zoster in a healthy adult population. After such a study, additional research will be needed to improve our knowledge of the immune mechanisms underlying VZV latency and reactivation.

Acknowledgment

We thank Susan Wood for her help in the preparation of this manuscript.

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